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BULLETIN 151: "BUDDY SAP"

A Preliminary Report Upon the Micro-organisms Occurring in Maple Sap and their Influence Upon the Quality of Syrup

By H. A. Edson[1]

SUMMARY

Maple sap as it occurs within the tree is free from bacteria and other micro-organisms.

As the sap flows from the tree it becomes infected, in the taphole, spouts and buckets, with wild yeasts, spores of molds, and countless numbers of bacteria. This infection becomes increasingly heavy with the advance of the sugar season and is the cause of the "souring" of sap.

Some of the types of hurt sap are caused by the action of specific groups of organisms, others may be caused by the collective action of many of the common forms.

Green sap and the resulting red syrup are not to be attributed to the swelling of the buds, but are caused by the development of a particular group of bacteria characterized by green fluorescence.

The dark color of the late run syrup is due entirely to the action of micro-organisms. If these are eliminated, as light colored syrup may be made from the last run as from the first run.

The flavor of the syrups is also seriously impaired by these agents. This injury often becomes pronounced before marked change in color is produced. "Buddy" flavors also appear to be due, at least in part, to the action of micro-organisms.

The quality of the product may be improved by: (1) keeping the spouts and buckets thoroughly clean; (2) using metal utensils in lieu of wooden ones; (3) gathering the sap at short intervals and boiling it in at once.

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[1]The author is indebted to Messrs. C. W. Fitch of the class of 1910 and C. W. Carpenter of the class of 1911 for faithful and efficient aid in field and laboratory work.

Introduction

Vermonters are familiar with the conditions under which maple sugar is produced, but for the sake of such readers as know little or nothing about "sugaring off," a brief description of the sap flow and of sugar making as practised in Vermont precedes the body of this article.[1]

Late in March, in this section, evidences of the coming spring appear. The nights are still cold and frosty but the days are genial and the temperature rises a few degrees above the freezing point. If, at this time, the trunk or limbs of certain species of the genus *Acer* are fresh wounded a sweet sap exudes. The Indians were familiar with this phenomenon before white men came; and had learned to collect, to concentrate and to make sugar from this sap. The early settlers[2] learned from them the essential steps which, in modified form, constitute the procedure followed in the maple sugar industry today.

According to modern practice the tree is tapped by boring a half inch hole 2 inches deep about 4 feet from the ground. A round, hollow spout or "spile" of wood or metal, upon which is suspended a bucket to catch the dripping sap, is driven into the hole. The sap flow is not continuous but is divided into short intermittent periods, technically termed "runs." It occurs only during the three or four weeks which immediately precede the unfolding of the leaf buds. Both its periodicity and its duration depend upon weather conditions. The sap is more likely to flow in the daytime than at night; and the more important runs are confined to what are spoken of as "good sap days." These occur only after the air temperature has remained below freezing for some time. If, following such a cold spell, the temperature rises materially above 32° F. a good run

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[1]For a comprehensive discussion, see Vt. Sta. Bull. 103 (1904).
[2]Garden and Forest 4, p. 171.

is likely to ensue. Excessive warmth and high winds check the flow. Freezing nights followed by moderately warm, cloudy days, and the absence of excessive sunshine and heavy winds, are the meteorological conditions which characterize the best sugar weather. So long as the air temperature remains essentially constant, whether warm or cold, little or no sap is obtained.

The buckets in which the sap is caught are made of wood, tin or galvanized iron; and, in the better works, are covered to keep out rain, snow and other foreign material. The sap is collected after each day's run and taken to the boiling house, technically known as the sugar house, where it is concentrated into syrup in large shallow pans over a roaring wood fire as rapidly as the capacity of the equipment will permit.

Maple sap is a sweet liquid containing a varying amount, averaging from 2 to 3% of saccharose, and, usually, traces of invert sugar. In addition to these carbohydrates it contains small amounts of proteids, of mineral matter, mainly lime and potash, and of acids, mainly malic acid. The sap of the earlier flows is water clear and transparent, and possesses a clean, sweet flavor. With the advance of the season however it undergoes a marked change. As the days grow warmer and night freezes are less severe and less frequent, the sap gradually becomes cloudy and discolored and unpleasant flavors develop. Such sap, while usually containing only the normal amount of acid, is popularly termed "sour." It rapidly deteriorates when stored even for a few hours. Several types of sour sap are recognized by sugarmakers, to which the descriptive terms "milky," "stringy," "red," and, particularly, "green" are commonly applied. Green sap is almost always secured just before the close of the season, when the leaf buds are ready to open. It is popularly believed that the swelling of the buds, associated with the renewal of vegetative activity in the tissues of the tree, is accompanied by a change in the composition of the sap within the trunk; and that the alteration in color and flavor are manifestations of this

change. The term "buddy" is universally used to describe this sort of sap.

The syrup made from late runs is much inferior to that derived from the earlier flows. "Last run" goods are often very dark in color and usually lack the delicate flavor possessed by the best syrups. Moreover, the quality of syrup varies markedly from year to year and these variations are seldom local in distribution. Such widespread fluctuations in quality are not accidental but of necessity must be associated with some fundamental cause or causes. It is conceivable that they may be related to weather conditions, either during the preceding summer or during the progress of the sugar season. It is known, moreover, that inferior products result from carelessness and lack of cleanliness in collecting and handling maple sap. Such procedures must occasion a great increase in the bacterial content of the sap, just as in dairying they entail serious bacterial contamination in milk. The proteid, carbohydrate and mineral contents of maple sap are sufficient to make it a fairly good medium for the development of bacterial life, provided suitable temperature relations are maintained; and the vital activities of large numbers of micro-organisms would presumably affect the flavor and quality of the syrup produced under such conditions.

Reflection upon these facts strongly suggests the possibility that micro-organisms may be associated with the inferiority of the maple output in all the cases cited, or that, indeed, they may be the direct cause of the troubles. Inevitably they must be present in the sap and it is to be expected that they would be more abundant toward the close of the sugar season than earlier, because the warmer weather would favor their increasingly rapid development and multiplication. It is conceivable that the "off" seasons may prove to be those wherein conditions foster this microscopic life to an unusual degree. More favorable temperature relations, or longer periods of incubation, or both, in "off" years

and in the latter part of the season, might reasonably be expected to promote the multiplication of organisms in the taphole, spout and bucket, thus producing heavy initial inoculation of the sap. Uncleanly methods would certainly result in this condition, and in any case, storing, even for a short length of time, would serve to increase the troubles due to the vital activities of microscopic organisms, particularly if the temperature of the storage house was considerably above the freezing point, as is apt to be the case.

Restating the proposition in the form of queries: What is the cause of the deterioration of maple sap? Is it due to changes in its composition occurring within the tissues of the tree as a result of the resumption of vital, protoplasmic activities and the renewal of vegetative vigor; is it due to the action of micro-organisms entering the sap after it leaves the vascular bundles of the trunk; or is it to be attributed to a combination of these causes?

The experimental work, upon which a preliminary report is submitted in these pages, was undertaken in an effort to determine so far as might be possible, the answer to these questions; and attention was first directed to a study of the micro-organic life in maple sap and its influence upon the quality of the sap and of the syrup produced from it.

The Effect of Micro-Organisms Upon the Keeping Quality of Sap

Early in April, 1907, sap was drawn from a tree under ordinary conditions, and placed in bottles of about 350 c.c. capacity, closed with cotton plugs. One-half of these bottles were heated at the temperature of boiling water in a steam chamber for a half hour on each of three consecutive days, to kill any living organisms which might be present. A very little sediment precipitated from the sap during this treatment; otherwise, it remained unchanged in appearance. Some two or three weeks after the last heating these bottles were capped with sealing wax to prevent

evaporation. They have been stored on laboratory shelves since that time without undergoing perceptible change. The flavor of the sap in bottles opened after 30 months was unchanged. The unsterilized sap on the contrary, promptly became turbid and developed one or another of the various types of so-called souring previously mentioned. Microscopic examination revealed the presence of multitudes of bacteria, as well as the spores of molds and yeasts.

DRAWING STERILE SAP FROM THE TREE

That these micro-organisms originated without rather than within the tissues of the tree seemed highly probable; but in order to demonstrate this point, and also to learn whether the preservation of the sterilized sap was due entirely to the absence of micro-organisms, or whether it was due in part to chemical changes induced by sterilization,—such for instance as the destruction of enzyms—an attempt was made to draw fresh sap in a sterile condition. Threads were cut upon each end of a straight piece of half inch gas pipe about 5 inches in length and a set nut was fitted on one end. This device was used as a spile. Threads were also cut on the larger end of a 9 inch tube, half inch in diameter at one end and three-eighths inch at the other, which was used as a delivery tube. The end of the spile not carrying the set nut and the larger end of the delivery tube were connected by a short piece of tightly fitting, flexible rubber tubing. This device constituted the conducting apparatus. Absorbent cotton was wound around the small end of the delivery tube until it was of such size as would enable it to fit tightly into the neck of the bottle used as a container. Care was taken to protrude the delivery tube an inch or more beyond the cotton. Above the cotton plug a sheet of cotton batting was fastened in such a manner as to form an umbrella like covering long enough to reach to the bottom of the bottle. When ready for use, the device resembled a limp and partially closed umbrella, the delivery tube placed in the mouth of the bottle being comparable to a hollow

umbrella handle, and the sheet of cotton to the cover. Prior to use, both ends of this apparatus were wound with sheets of absorbent cotton and the entire device placed in the autoclave and sterilized by heating for 30 minutes under a pressure of 10 pounds of steam. The collecting bottles were plugged with cotton and sterilized in the autoclave. The bit used later in tapping the tree as well as the shave for removing the outer bark were sterilized and immersed in boiling water, in which they were kept until the instant of use. The tree was shielded from the wind during the process of tapping. The rough bark was broken away with a hammer and, by the use of the sterile shave, the corky material was removed until only a thin portion remained. The shave employed was dipped in boiling water after each stroke; and as an added precaution the area thus prepared was finally washed with formalin, and then dried by the heat from an alcohol torch. Then a hole was bored to a depth of 2.5 inches, using the sterilized bit. An assistant removed the free end of the 5 inch length of gas pipe from the cotton covering at the instant the bit was removed from the tap hole, and inserted the spile into the tree. A pipe wrench was used to turn the set nut firmly against the trunk. Great care was taken in this operation to eliminate contamination from the hands, the air or the tree bark. With equal precaution the outer wrapping of cotton was removed from the delivery tube, and the end of the conducting apparatus thus freed was inserted into the neck of the collecting bottle, so as to bring the cotton plug into position. The bottle was then placed in a rack upon a support prepared for it, and protected from accident by a shield. The work of tapping and setting up the apparatus was carried out before the sap started and while the tree was still frozen, in order to avoid the disadvantages which would result from the presence of liquid sap. As fast as the collecting bottles were filled they were removed and others were subsituted. The exchange was carried out with extreme care in order to prevent contamination of the interior of the delivery tube or of either of the bottles. The sterile cot-

ton plug of the fresh receptacle was transferred to the full one at the same time that the delivery tube was changed. The operation was carried out under a shield of sterilized cotton so as to prevent as far as possible contact with currents of unsterilized air.

The first tree was tapped March 18, 1907 at 8.30 a. m., while it was frozen. There was no flow of sap from this tree until the afternoon of March 25, when two bottles were obtained in sterile condition. On March 30 and on April 3 other trees were tapped in the same manner. Sap was obtained in several bottles in which no development of bacteria, yeasts or molds occurred. In a certain percentage, however, as was to be expected, growth developed; but this is to be attributed to some slip in the technique, such as contamination incident to changing bottles or infection in the tap hole made possible through expansion and contraction accompanying changes of temperature. The sap in which no contamination occurred has remained unchanged in appearance and in flavor up to the present time, (February, 1910), an interval of nearly 35 months.

These results seem to justify the opinion that the "souring" of maple sap is in no degree a process of auto-decomposition, but, rather, one to be attributed entirely to the action of living organisms.

ISOLATION OF ORGANISMS

The contents of such bottles of sap as did not keep, that is to say which showed clouding or "souring," were examined for bacterial contamination. Organisms were found to be present in abundance. Pure cultures were obtained by plating upon ordinary nutrient agar and also upon a synthetic medium made up as follows:

Water	1000	parts.
Dextrose	100	"
Peptone	20	"
Ammonium nitrate	2.5	"
Magnesium sulphate	5	"

Potassium nitrate	2.5	parts.
Potassium phosphate	2.5	"
Calcium chlorid	0.1	"
Agar	15	"

The first of these media, the nutrient agar, is designed especially for the cultivation of bacteria, while the second, the synthetic medium, inhibits the growth of most bacteria, but is very favorable to the development of yeasts and molds. A large number of plates were poured with each of these media and an attempt was made by examining and comparing the appearance of the colonies which developed to determine the predominant organisms. Pure cultures of these dominants were secured and reserved for further study. Various samples of normal sap drawn under ordinary conditions were examined for micro-organisms in a similar manner and pure cultures of the more common ones secured. Among the organisms thus obtained were green molds belonging to the genera *Penicillium* and *Eurotium;* a few wild yeasts; and many of the saprophytic bacteria, among which those producing fluorescent colonies were the most common type.

HURT SAP

Shortly before the opening of the sugar season of 1908 a letter was sent to a few representative sugarmakers in different sections of the state requesting samples of any unusual sap which might be found in the buckets during the coming season. Especially were samples of stringy sap, sour sap, or other abnormal material solicited. Among the considerable number of samples thus secured, all of the more common forms of hurt sap were found, such as stringy sap, red sap, and green sap, as well as milky forms of so-called sour sap. As soon as each sample reached the laboratory it was immediately subjected to microscopic examination, and was plated upon both the synthetic and the nutrient agar. Cultures of the more abundant organisms were secured, and replated as many times as was necessary to insure purity, and the final result retained for further study.

Stringy sap: One of the samples received was decidedly milky and stringy, and possessed a strong characteristic odor, suggesting yeast. The microscope revealed great swarms of actively motile bacteria, but no yeasts were discovered, either in the sediment or in the liquid portions. Plating served to develop bacterial colonies only, and these were practically all of one type. Pure cultures of this organism were isolated April 24, 1908. Subsequent inoculations have shown it to be capable of reproducing the peculiar condition occurring in the sample submitted. Its development was accompanied by mild acid formation and slight gas production. The cultural and morphological characters of this organism, which is believed to be a new species, have been studied and the results are to appear in a subsequent bulletin.

Green sap: Under this head are included all those types of hurt sap which possess a greenish or greenish-brown color. A large number of the samples submitted were of this character. Microscopic examination showed them to contain micro-organisms, frequently of several different types. The colonies developing on poured plates were always of more than one type, and in many cases included yeasts and molds as well as bacteria, though the latter were very much more numerous. The type of colony most frequently secured was of a green fluorescent character. In many cases 90 percent or more of the colonies were of this type. Critical studies of such bacteria of this class as have been isolated are under way, but the results are not yet ready for presentation. The organisms are all members of the *Pseudomonas* group, and appear to be more or less closely related to *Pseudomonas fluorescens*. Both the liquifying and non-liquifying types are included. None of these organisms ferment carbohydrates nor do they produce acid, either in maple sap or in the artificial carbohydrate media tested. Inoculation experiments reported on page 499 show that the different members of this class thus far studied are capable of producing green

clouding of maple sap. The indications are that they feed upon the traces of proteids present in the sap, while they leave the sugars unchanged, or at least unfermented.

Red sap: A few samples contained a reddish brown sediment, which, upon agitation, gave to the whole volume a red color. This material was especially rich in yeasts, though the fluorescent bacteria already described were also abundant. The red color appeared to be due to a development of certain yeast-like organisms which formed red colonies. Associated with them were other yeasts or yeast-like bodies which developed gray colonies. Both of these organisms have been found to occur very commonly in the saps which have been studied now for several seasons. They appear to be widely distributed in the sugar woods of the state. Neither of them seems to be capable of fermenting the common sugars, dextrose, lactose and saccharose. Inoculation experiments with these organisms are reported on page 502.

Milky sap: Certain samples submitted had the characteristic cloudy appearance and unpleasant, slightly bitter flavor of the green types, but in place of the greenish yellow tinge showed a pale milky hue. A relatively large proportion of the colonies of organisms isolated from those saps were of a pearly white character, distinctly different from those already mentioned. Inoculation experiments were carried out with these organisms in the same manner as with the other groups (page 500). Acid and gas production were both absent in cultures grown in sap or in any other of the carbohydrate media employed during the preliminary studies.

Molds: Constantly associated with other organisms in sap there occurred a certain number of mold spores. These were seldom found in any great number, but because of their universal appearance upon the plates, pure cultures were secured. These belong to the genera *Penicillium,* and *Eurotium,* the com-

mon green molds. Inoculation experiments indicate that these molds may be of some importance in causing the inversion of sugar. See page 502.

FIELD STUDIES ON THE NUMBER AND CHARACTER OF MICRO-ORGANISMS IN MAPLE SAP

In connection with the 1909 field studies (reported on pages 496-507), as much attention as circumstances permitted was given to making further observations on the numbers and character of micro-organisms present in sap, using material secured from several sugar places at various intervals during the season. The time available for this study was so limited that only a few determinations could be made; but the results obtained are so significant that it seems best to present them in this preliminary report. It is planned to continue studies of this nature as opportunity permits.

The predominating organisms found during the earlier days of the sugar season belonged to the yeast-like group, while bacteria were relatively few in numbers. As the season advanced these conditions were reversed. Plates were poured on March 2, employing the synthetic agar upon which most bacteria do not develop, and also a nutrient agar adapted to the growth of yeasts, molds and bacteria, but especially to that of the latter. The number of organisms developing on synthetic agar in the material obtained from four different trees was 610, 500, 140, and 220 respectively, per c.c. These figures represent the yeast and mold content of the saps. The counts from the same sap plated upon nutrient agar showed the presence of 900, 1000, 140 and 220 organisms per c.c. respectively. The colonies developing upon nutrient agar included many yeasts as well as bacteria, hence it is evident that the bacterial content of these saps was low. It is also significant that few fluorescent colonies were found. Many of the series of agar plates show none of this group.

Trials made April 4 gave the following results: The sap from tree 1, on synthetic agar developed 44,000 colonies per c.c., chiefly of two types, the gray and red yeasts previously described. On nutrient agar 66,600 colonies appeared, a few of which were of the green fluorescent type. The sap from tree 2, on synthetic agar, showed a count of 1100 colonies, chiefly molds. On April 6 the sap from tree 1 showed a development of colonies on synthetic agar so numerous that they could not be counted. Its content, however, must have been over a million per c.c. On plain agar 200,000 colonies developed. Tree 2 on the same date on synthetic agar, gave a count of 2,700, and on plain agar a count of 2,600 per c.c. The types of colonies developing were similar in character to those reported from the same trees two days earlier. Attention is called to the fact that an incubation interval of six days was required for the development of the colonies reported. Upon one of these plates a few colonies of the green fluorescent type of bacteria appeared within 36 hours, but there was no sign of other colonies until the fourth day. The majority of the organisms in this sap, therefore, belonged to slow growing types, and their influence might be almost imperceptible under ordinary conditions.

Plates were poured April 12 with a view of determining the number of bacteria present; but notwithstanding the use of a dilution of 1 to 100, it was entirely impossible to count the colonies which developed. They made a rapid growth and, significantly, fluorescence was detected in the medium even before visible colonies had developed. The majority of the colonies must have belonged to the green fluorescent type. Yeast colonies were found on a few of the plates, but only in small numbers.

On April 15, plates were poured from the sap of five trees which were running sour. No attempt was made to determine the number of bacteria present, since facilities for making the proper dilutions were not available. The sap from one of these trees was of the milky type, while that from the other four trees was of a yellowish green or brown type of souring. The pre-

dominating organism in the milky sap developed pearly white colonies. The predominating organisms in the green samples were nearly all of the blue-green or yellowish-green fluorescent type. The number of fluorescent bacteria so far exceeded that of the other types as to closely approach a condition of pure culture. On the day following samples from other trees in various sugar places where the sap was "running green in the buckets" were plated, to see if these results would be confirmed. Without exception it was found that the green fluorescent bacteria were present in enormous numbers. These observations suggest that the green color may be due entirely to the presence of bacteria rather than to the swelling of the buds with which it is so often associated. Additional evidence was secured upon this point in the experiments reported upon page 504 under the heading "late run syrup."

INOCULATION EXPERIMENTS

The results of the earlier observations suggested so clearly the relationship between bacterial infection and abnormality of maple products, that it seemed advisable to conduct inoculation experiments to determine definitely its influence upon their quality.

The sugar orchard in which the field experiments were carried out is situated upon a western slope, but is exposed to north winds. The soil for the greater part is wet, and the orchard has had the reputation of producing a grade of syrup of medium standard both in flavor and color.

One hundred trees were tapped on March 24, 1909, galvanized iron Warner spouts and clean tin buckets with japanned sheet iron covers being used. The sap used for inoculation was collected late in the afternoon in clean tin cans, such as are commonly used for transporting milk, and carried directly to the field laboratory. It remained in the cans over night at a temperature just above 32° F. The next morning it was placed in new tin buckets in several 16-quart portions. One of these was

reserved for a control, while each of the others was inoculated with 70 c.c. of 24-hour old cultures of bacteria or other organisms, as the case might be. At the same time 70 c.c. of sterile culture media of the same composition was added to the control. The inoculated saps and the control were placed in a room where the temperature varied during the day from 65-70° F. dropping at night to about 40° F. After three days the various portions were made into syrup, each of the several saps being evaporated in a bright sugaring-off pan on a kitchen stove until condensed to the volume of about one quart. This was then transferred to a white, agate-ware basin, and evaporated as rapidly as possible until the proper concentration, as indicated by a thermometer, was reached. The process, from cold sap to syrup, required about an hour and a half for its completion. The syrups, each about one pint in volume, were transferred at once to glass jars and sterilized by heating in a steam chamber at the boiling point of water for one hour and sealed. They were then stored in the dark until such time as they could be subjected to chemical analysis and scored for flavor and color. In order to eliminate the personal element the assistance of a commercial expert, unfamiliar with the history of the various samples, was secured to score the goods. Three grades of color and as many of flavor, were recognized, which may be considered to correspond respectively to good, poor and very poor. In addition to this general classification an attempt was made to select the sample or samples which in the opinion of the judge represented the finest and the poorest products. These special cases will be noted in the discussion of the respective series in which they occur.

A series of color charts was also prepared. These, while they do not represent exactly the true shades of the syrup, are at least comparative and give an adequate idea of the relative colors. It is greatly to be regretted that it is impracticable to reproduce these tints in this bulletin.

While it is true that pure cultures were not maintained during the progress of these inoculation experiments yet it is be-

lieved that, in the case of the bacterial cultures at least, a sufficiently heavy inoculation was produced to insure an overgrowth of the specific organisms under investigation. While the possible influences of other organisms naturally present must not be overlooked, it seems safe to conclude that the several results obtained are due largely to the several organisms artificially introduced, for the controls were subjected to the same natural inoculations as were the other samples.

The organisms used in these inoculation experiments were:

A. Six different cultures selected from the green fluorescent group.

B. Two different cultures selected from the other types of sour sap, and one slime producing organism obtained from a sample of stringy peas.

C. The stringy sap bacillus.

D. Five undetermined yeast cultures and one green mold.

With the exception of the stringy pea organisms the cultures employed were obtained from maple sap as already explained. It was considered important that inoculation experiments be conducted upon the first run. This, however, proved impossible, since only about 20 gallons of sap were obtained from the trees on the first day. That obtained on March 24 was used as far as it would go. March 25 and 26 were stormy days, but a light run occurred March 27. Care was taken on that morning that all buckets should be empty and clean, so that the sap then gathered should correspond as closely as possible in every way to that obtained in the first run March 24. The inoculations reported under series A, B, and C were carried out with this material. Series D was carried out on sap obtained on April 1 in the same manner as already described for the first run,[1] and inoculated April 2.

[1]The numbers employed in the following discussion have no reference to the order in which the samples were produced. They are those used in the laboratory in connection with the scoring and analysis, and were assigned as a matter of convenience to bring related samples into consecutive numerical relation.

SERIES A. GREEN SAP BACTERIA

Six of the typical organisms producing green fluorescent colonies were selected as representatives of the hundred or more cultures of this class which had been isolated. These will be discussed under the numbers 7-12, inclusive. Within six hours after inoculation the saps numbered 8 to 12 inclusive developed a greenish color which was particularly noticeable on looking down through the sap in a good light which was reflected back from the bottom. A similar appearance developed in 7 at a later hour. All of the samples became turbid and displayed a yellowish green fluorescence in some lights. At the end of the incubation period the samples were titrated with N/100 sodium hydroxid to determine the production of acid if such occurred. The results in all cases agreed very closely with those obtained with the check, both at the beginning and at the end of the incubation period. Fermentation tubes of sap inoculated with these several organisms and held under observation along with the material to be evaporated showed no signs of fermentation.

The syrups obtained from the saps thus inoculated presented a striking contrast with the control syrup (No. 2). All were very dark colored, with a noticeable admixture of red. They were all ranked as second grade goods—in color—save one, No. 10, which was deemed to be of first grade. This sample, while much darker than the control, was somewhat less muddy in appearance than were the rest of the series. The flavors were injured even more than were the colors. No. 7 was scored third grade, ranking with one other as the poorest syrup made during the season. The rest of the series were deemed to be of second quality. The control syrup, (No. 2) was scored as first quality, both in color and flavor. It was in every way a gilt-edge product, being the best single sample obtained during the season. Chemical analyses showed that the reducing sugar content of the inoculated syrups were slightly augmented, although the figures obtained are below the average for market sugar.

SACCHAROSE AND REDUCING SUGAR CONTENTS OF SERIES A, CALCULATED TO A DRY MATTER BASIS

Treatment	Sample number	Saccharose	Reducing sugar
Control	2	95.41	0.97
Inoculated with organism XXXIII	7	96.79	1.55
Inoculated with organism XXXVI	8	96.73	2.09
Inoculated with organism L	9	96.54	1.56
Inoculated with organism LIII	10	97.16	1.44
Inoculated with organism LVI	11	96.16	1.64
Inoculated with organism 5	12	97.14	1.15

SERIES B. MILKY SAP BACTERIA

Sap samples 13 and 14 were inoculated with organisms obtained from milky, "sour" sap, while No. 15 was inoculated with a culture obtained originally from stringy peas. The type of souring produced was characteristic and similar in all cases, except that the foreigner was capable of very slight acid production, accompanied by but little gas formation as indicated by tests in a Smith tube. The inoculated sap became cloudy within the first few hours and soon developed a milky appearance. No marked odor was detected, but the flavor was unpleasantly changed, a bitter but not acid taste developing.

The syrups of this series, while darker than the control (No. 2), were very much lighter than were those of series A. All were placed in the first class as regards color; yet notwithstanding this fact the samples all present an unattractive appearance, because of their muddy and viscous character. No. 13 in particular developed this property to a marked degree. While the sap just before concentration showed no signs of stringing, this syrup possessed sufficient viscosity to enable it to be readily drawn into strings several inches long. The cloudy appearance remained undiminished after three months of sedimentation, during which time the jar was not disturbed. The flavor of the entire series, moreover, was very seriously impaired, being so unlike the natural maple taste that one unfamiliar with the history of the syrups would be inclined to question their genuine-

ness. Syrup 13 was rated as of second grade in flavor and the others as of third grade. Their reducing sugar contents were small save in 15. The relatively large amount present in this syrup is perhaps the result of the acid produced during incubation.

SACCHAROSE AND REDUCING SUGAR CONTENTS OF SERIES B, CALCULATED TO A DRY MATTER BASIS

Treatment	Sample number	Saccharose	Reducing sugar
Control	2	95.41	0.97
Inoculated with XXVI	13	95.61	0.78
Inoculated with XXX	14	95.86	0.77
Inoculated with I	15	95.71	2.23

SERIES C. STRINGY SAP BACTERIA

Within a few hours after the introduction of a 70 c.c. culture of this organism into the sap, the material became cloudy and then milky. As the incubation proceeded a deep milky white color developed, which rendered even thin layers opaque. It became viscous and ropy, so that in turning it from one vessel to another the weight of the stream continued to syphon the sap over the edge of the dish even when the rim was raised to somewhat above the level of the liquid contained therein. It developed the characteristic sour, disagreeable, yeasty odor of the ropy sap from which the organism was obtained. The taste became bitter and unpleasant in the extreme. Fermentation tube tests showed moderate gas production, 15 to 25 percent of the closed arm being filled during the period of incubation, the gases produced being carbon dioxid and hydrogen. Titration tests before concentration of the sample showed that acid production was in progress, 50 c.c. of the sample requiring 85 c.c. of N/100 sodium hydroxid to neutralize it against phenolphthalein without heating. An equal quantity of the control sap was neutralized by 1 c.c. of the same alkali. The vapor evolved during the concentration was extremely unpleasant, and was sufficiently noticeable to attract the attention of a sugar-maker who was passing the house where the field laboratory

was established. Although he was entirely unaware of the nature of the experiments, the steam borne to him through an open window elicited the remark, "It smells like the last of sugaring." The syrup obtained was similar in color to that obtained in series B., being light colored but muddy in appearance. After standing upon the laboratory shelves for four months the muddy character was still very marked, showing that it was not easily removed by sedimentation. While rated as of first grade in color it was easily third class in flavor.

SACCHAROSE AND REDUCING SUGAR CONTENTS OF SERIES C, CALCULATED TO A DRY MATTER BASIS

Treatment	Sample number	Saccharose	Reducing sugar
Control	1	95.41	0.97
Inoculated with LXXXVII	16	96.12	1.72

SERIES D. YEASTS AND MOLDS

The inoculation experiments with yeasts and molds, conducted on samples 17-23 inclusive, were less satisfactory than were those with other organisms, because of the relatively high initial contamination of the sap employed. As previously noted, the sap was drawn several days later than that used in the first series. At the close of the incubation period the control was beginning to show signs of change from the continued growth of the organisms naturally present. An examination of the slime produced on the sides and bottom of the buckets of the inoculated sap showed the presence of great numbers of bacteria and comparatively few of the specific yeasts or molds introduced. Moreover, the type of souring produced was very similar to that described for series A and B. Tests for acid formation and gas production gave negative results, which were later confirmed in pure culture experiments. Observations confined to an examination of the sap during the incubation period would have led to the conclusion that the yeasts and molds employed developed too slowly in the maple sap to be of importance. On the other hand the bacteria appeared to develop better in the samples in-

oculated with yeasts than in the control. This cannot be due to food matter introduced with the inoculation material, since an equal quantity of sterile medium of the same composition was added to the control. It is possible that some stimulating relation exists between these organisms and the bacteria. If this should prove to be the case, it will follow that the yeast and molds are important factors in the spoiling of sap, because of the impetus they give to bacterial development, as well as because of any changes which may be induced by their own metabolism. Upon concentrating the materials, however, a surprising difference was noted between the control (No. 5) and the other samples of the series. This difference can be accounted for only by the artificial inoculation. The control, while dark in color and of a less delicate flavor than the control of the first three series, was yet a good product, justly scored as first grade. Numbers 17, 20 and 21 of the inoculated samples were rated as first grade in color, the other four as second grade. In flavor, numbers 19 and 23 were rated as third grade while the remaining five were scored as of second grade. No. 19 was regarded with another of an earlier series as the poorest samples produced during the season. The most significant fact brought out by the chemical analyses is the increase in reducing sugars caused by the introduced organisms.

SACCHAROSE AND REDUCING SUGAR CONTENTS OF SERIES D, CALCULATED TO A DRY MATTER BASIS

Treatment	Sample number	Saccharose	Reducing sugar
Control	5	95.37	1.58
Inoculated with 24	17	93.34	2.53
Inoculated with 25	18	95.30	3.01
Inoculated with LXIV	19	95.73	2.61
Inoculated with LXII	20	93.26	2.98
Inoculated with LXXV	21	92.76	3.47
Inoculated with LXXIX	22	91.65	3.12
Inoculated with all of above six organisms	23	93.28	4.09

LATE RUN SYRUP

The inoculation experiments as well as the laboratory and field studies showed clearly that bacteria are associated with poor quality in maple sap, and that anything which tends to favor the growth of these organisms is detrimental to the sugar-makers' product. It remained to determine whether the darker color and ill flavor of the late run syrup is to be attributed wholly or only in part to the activities of micro-organisms.

On April 12 it was noticed that the sap outputs of two trees were beginning to show signs of souring, notwithstanding the fact that care had been taken to empty the buckets at least once a day. In order to determine whether this sour condition was due to the development of the micro-organisms in the spouts and buckets, or whether it was to be attributed, in a measure at least, to sap changes within the trunk, both of these trees were re-tapped a second time on a level with the old hole and about five inches from it. New spouts and clean buckets were hung at the new tap holes. This was done at about 5 p. m. The sap was emptied from the old buckets, which were not washed, but were returned to their original places and left until noon the next day. The two lots of sap, the older and the fresher, each treated as a unit, were then transferred to the field laboratory in clean pails and examined at once as to appearance and flavor. The sap gathered from the old tap holes was slightly cloudy, of a yellowish cast and possessed a decidedly "buddy," bitter taste. The sap obtained from the same trees at the same time but from the new tap holes was perfectly clear and possessed no unpleasant taste. It was apparently entirely normal in every respect. These saps were made up into syrup at once. That from the old tap holes (No. 24) was muddy and off color, although not so poor as much of the material obtained by inoculation early in the season. While it was scored as first grade in color it was just on the border line between first and second grade. It was rated second grade in flavor. The syrup made from the new tap hole

(No. 6) was of very light color and excellent in flavor, being scored as first grade.

On the evening of April 13, sap was gathered again from the trees above described and that drawn from the newer tap holes combined with that obtained from two other trees which had been retapped earlier in the day. In order to determine the effect of a short period of storage, the material was left over night in new tin buckets which had been thoroughly scalded just before use. The sap from the old tap hole by this time had become badly clouded and had a marked bitter taste, while that from the new tap holes showed neither clouding nor change of flavor. The syrup from the old tap holes (No. 26) had the color of poor molasses with a flavor to match, being scored as third grade in color and a poor second grade in flavor. It was characterized as entirely lacking in maple flavor. The syrup from the new tap hole (No. 3) was of light amber color and good flavor, being rated as a good first grade article.

On April 16, three trees which had been running green sap were retapped in the same manner as just described. The tappings were made in the early morning just as sap was starting. Late in the afternoon of the same day the saps for both old and new tap holes were collected and transferred to the field laboratory where they remained over night at a temperature just below 32° F., as evidenced by the thin ice skim which formed. The following morning the two batches were made into syrup. That from the old tap hole (No. 25) was of dark color and of a decidedly "buddy" flavor, but scored a second grade in both respects. The syrup obtained from the new tap hole (No. 4) was of light color and of very good flavor, being one of the best samples produced during the season. While the flavor was very slightly inferior to that of the control of the first run, the color was fully as light, and it would easily pass for a gilt edge product.

SACCHAROSE AND REDUCING SUGAR CONTENTS OF SYRUPS MADE FROM LATE RUN SAPS, CALCULATED TO A DRY MATTER BASIS

Treatment	Sample number	Saccharose	Reducing sugar
Control, first run	2	95.41	0.97
New sap kept	3	96.02	0.61
New sap	4	96.42	0.51
New sap	6	95.19	0.82
Sour sap	24	95.27	2.76
Sour sap	25	95.05	3.13
Sour sap kept	26	92.56	3.55

SUMMARY OF ANALYTICAL RESULTS

For the sake of convenience in making comparisons the following summary of analytical results is given. The ratio column contains the results obtained by dividing the saccharose content by the reducing sugars, and is intended to show the ratio of the two. The higher the number, the lower the ratio of reducing sugar to saccharose. The samples included in this table have all been previously described except No. 1. Sample No. 1 belonged to the same batch of sap and was handled in exactly the same way as was sample No. 2, except that it was condensed to a syrup under conditions that required six hours for its evaporation. There was practically no difference in the flavor of the two samples, but the slowly evaporated material was slightly darker in color, a result presumably due to some chemical change which took place during the boiling. Attention has been called to the fact that the remaining 25 samples were concentrated under as nearly uniform conditions as could be obtained, so that the time required for evaporation did not in any case vary more than 10 or 15 minutes from an hour and one-half.

Description of sample	Sample number	Saccharose	Reducing sugar	Ratio	Color	Flavor	
Controls.....	1	96.84	1.07%	90	1	1	
	2	95.41	0.97	98	1	1	cf. with A, B, C
	3	96.02	0.61	157	1	1	cf. with 26
	4	96.42	0.51	189	1	1	cf. with 25
	5	95.37	1.58	60	1	1	cf. with D
	6	95.19	0.82	116	1	1	cf. with 24.
Series A.....	7	96.79	1.55	62	2	3	cf. with No. 2
	8	96.73	2.09	46	2	2	
	9	96.54	1.56	62	2	2	
	10	97.16	1.44	67	1	2	
	11	96.16	1.64	59	2	2	
	12	97.14	1.15	84	2	2	
Series B.....	13	95.61	0.78	122	1	2	
	14	95.86	0.77	124	1	3	
	15	95.71	2.23	43	1	3	
Series C.....	16	96.12	1.72	56	1	3	
Series D.....	17	93.34	2.53	37	1	2	cf. with No. 5
	18	95.30	3.01	32	2	2	
	19	95.73	2.61	37	2	3	
	20	93.26	2.98	31	1	2	
	21	92.76	3.47	27	1	2	
	22	91.65	3.12	29	2	2	
	23	93.28	4.09	23	2	3	
Old tap hole...	24	95.27	2.76	35	1	2	cf. with 6
	25	94.05	3.13	30	2	2	cf. with 4
	26	92.56	3.55	26	3	2	cf. with 3

CONCLUSIONS

A consideration of the data reviewed in the previous pages and of the discussion thereof would seem to justify the following preliminary conclusions.

While maple sap within the vascular bundles of the tree is free from bacteria, yeast, and molds, it becomes infected therewith in the tap holes, spouts, and buckets. These micro-organisms are important factors operating against successful sugar making. There are specific types of poor sap which are caused by specific organisms. There are other types of poor sap which

may be produced either by the independent action of individual species, or by the collective action of a large group of the common saprophytic organisms. The common green sap is to be attributed to the action of green fluorescent bacteria, rather than to the swelling of the buds. The term "buddy" as applied to it is a misnomer. Such material might be described more accurately, though perhaps less elegantly, as "buggy." The dark color of syrup made from late run sap, which has been protected from the entrance of extraneous materials, is due entirely to the action of micro-organisms. The buddy flavor is likewise due in a large measure to the same cause. In the opinion of the author the very slight difference in flavor between the sample produced from the fresh tap hole during the last run, and the sample produced from the fresh tap hole during the first run, could easily be accounted for by the action of micro-organisms growing in the sap during the few hours while it was being collected. The much warmer temperature would certainly result in a very much more rapid development of organisms in the sap drawn on April 16 than in that drawn late in March. However, it is possible that some slight change in the composition of the sap itself may take place within the tree, and final judgment upon this point must await the results of further studies which will be carried out as opportunity permits. In any event, it is an established fact that the warmer weather of the last days of the sugar season affords conditions which are especially favorable to the rapid multiplication of those organisms which have become established in the spouts and buckets. Under ordinary conditions late in the season, they are present in the sap in enormous numbers and result in the production of a very dark type of maple syrup which is also of inferior flavor. By eliminating their action the development of the dark color can be prevented, and nearly all, if not all, of the buddy flavor can be avoided.

Remedial measures

Practical remedial measures must be based upon efforts to minimize the contamination with micro-organisms and to restrict the period of their action to the shortest possible time. The lower their content and the shorter their period of growth, the better the product. As in dairying, cleanliness must be the watchword of the producer of superior goods. Clean spouts, clean covered buckets, and clean holders are necessities. The use of metal utensils is to be preferred to the employment of wooden ones, because the latter material affords organic matter upon which organisms may develop. Wooden utensils are moreover less readily cleaned. Covered buckets are preferable to open ones, because they not only keep out rain and snow, but they prevent the entrance of bits of falling bark, decayed wood, and other inert matter. Such material is always heavily charged with bacteria and other organisms; so that in addition to the coloring matter carried in the refuse itself, agents are introduced which further discolor the product through their vital activities. In the Ohio valley, where the sugar season is somewhat longer than is the case in Vermont, the practice of retapping is much in favor. The trees are first tapped with a small bit, then after the season becomes more advanced, the holes are rimmed out with the usual size rimmer. This not only reopens the wound which has become partially dried out, but also frees it from much of the accumulated growth of micro-organisms. The practice of gathering the buckets and washing them at intervals is recommended by certain producers who are interested in turning out only a gilt edge product. This is more easily done if extra buckets are available so that clean ones may be hung at the spouts at the same time the old infected ones are gathered. Buckets in different parts of the orchard may thus be changed on different days. The time between runs affords a favorable opportunity for the work of washing. Flushing out the tap holes and spouts with clean water would doubtless aid in the efficiency of the treatment. Whether the increase in the

market value of the syrup produced in this way will be sufficient to justify the additional labor and expense involved is a question which must be considered by the sugar maker, and decided by him according to local conditions and individual tastes.

The practice of storing sap is one to be avoided whenever possible. Modern evaporators not only make long periods of boiling unnecessary, but they make it possible to concentrate the runs day by day as they occur. These are doubtless two important factors contributing to the improved quality of evaporator syrup as compared with that produced by older methods. When storing is resorted to, the temperature of the tank should be kept as low as possible, because the lower the temperature the slower the micro-organisms develop. Holders should be located without rather than within the boiling house, where the heat of the pans will not influence their temperature.